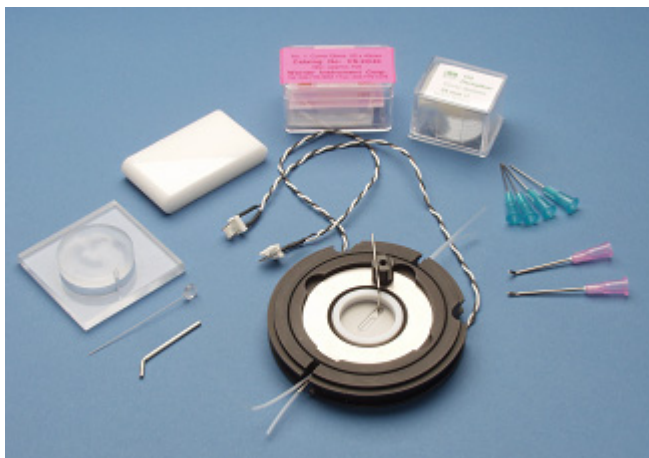


WARNER IMAGING CHAMBERS

A feature in common with all Warner imaging chambers is the use of a glass coverslip as the floor of the chamber. When viewed with an inverted microscope, images are visualized through a glass thickness of 0.13-0.17 mm.

THE RC-50

The **RC-50** is a low profile, horizontally mounted Ussing chamber incorporating special features for confocal imaging. This unique chamber is designed to be used with both inverted and upright microscopes, and can be configured to operate in either open or closed bath modes.



In the closed bath configuration, a #1 glass coverslip forms both the top and bottom of the chamber allowing it to be used with both inverted and upright microscopes. The chamber platform (basolateral side) is beveled to permit maximum access by the microscope objective to the bottom coverslip. The basolateral viewing aperture is 17.7 mm in diameter and the apical viewing aperture is 22.8 mm. Resistive heating elements are integral to the chamber platform and are compatible with Warner's **TC-324B** and **TC-344B** Heater Controllers.

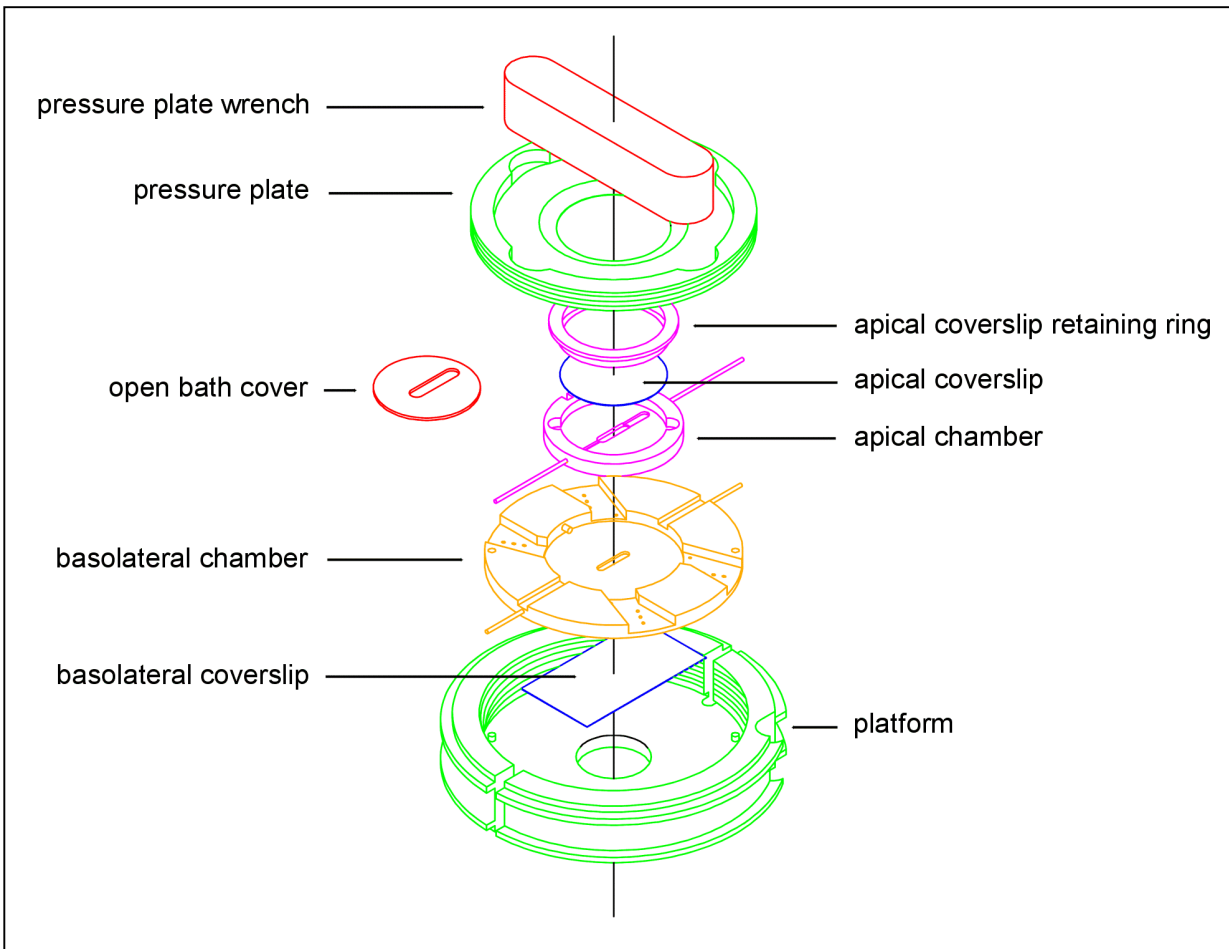
The **RC-50** comes complete with:

- chamber
- open bath cover
- platform
- basolateral chamber coverslip mounting tool
- 25 mm round glass coverslips (#1, 100 ea.)
- 22 x 40 mm glass coverslips (#1, 50 ea.)
- coverslip removal tools (2 ea.)
- 23 gauge needles (4 ea.)
- aspirator tubes (1 ea. large and small)
- PE-90 tubing (10 ft.)
- PE-160 tubing (10 ft.)
- perfusion tube cleanout tool

ASSEMBLY

The general procedure for assembling the **RC-50** is to first attach the BASOLATERAL COVERSIP to the BASOLATERAL CHAMBER. This is followed by submerging the BASOLATERAL CHAMBER and stretching the epithelia. Next, the APICAL CHAMBER is assembled, mounted and sealed. Finally, solution delivery tubing is placed and the chamber is placed into the PLATFORM. At this point you are ready to mount the **RC-50** onto your microscope, usually via a stage adapter.

NOTE: Refer to the schematic on the next page as a guide while assembling the **RC-50**.

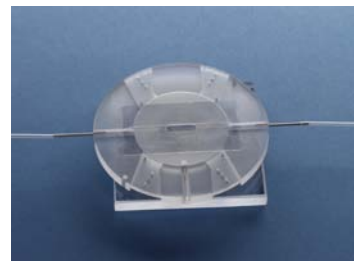


Assemble the Basolateral Chamber

1. Begin by locating the BASOLATERAL CHAMBER COVERSIP MOUNTING TOOL (shown to the right).
2. Place the BASOLATERAL CHAMBER upside down onto the MOUNTING TOOL. Note that the tool is 'keyed' to accept the associated pin in the BASOLATERALCHAMBER.
3. Locate the 22 x 40 mm indentation on the underside of the BASOLATERAL CHAMBER. Using a small paintbrush (size 0 or 00 works well), apply a thin layer of vacuum grease to this indentation.

NOTE: Avoid getting grease into the perfusion ports or flow channels which run from the ports to the oval aperture.

4. Press a clean 22 x 40 mm coverslip into the indentation to evenly distribute the grease. Remove the coverslip and dispose. Clean any excess grease from around the aperture and flow channels, and secure another clean 22 x 40 mm coverslip into place. A properly mounted BASOLATERAL COVERSIP is shown to the right.



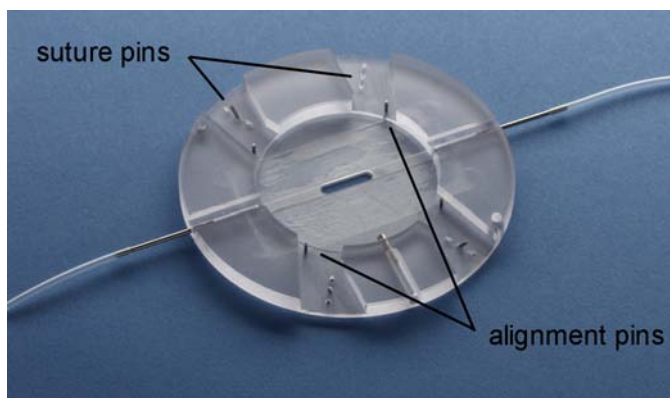
- Remove the chamber from the BASOLATERAL CHAMBER COVERSIP MOUNTING TOOL and place it upright on a clean surface. Using the paintbrush, apply a thin coating of vacuum grease to the circular area surrounding the oval aperture in the bottom of the BASOLATERAL CHAMBER.

NOTE: Avoid getting vacuum grease into the oval aperture or you will need to clean and re-assemble the BASOLATERAL CHAMBER.

Mount the Tissue

- Submerge the assembled BASOLATERAL CHAMBER into a previously prepared 6" Petri dish which has been bottom-coated with a layer (~ 1/4") of Sylgard. Use a sufficient quantity of working buffer to completely submerge the chamber.
- Place your prepared tissue over the aperture in the BASOLATERAL CHAMBER and stretch it into position using suture lines.

NOTE: Slots have been milled into the BASOLATERAL CHAMBER to facilitate placement and stretching of the tissue. SUTURE PINS are provided to act as anchor points for the suture lines. If desired, the ALIGNMENT PINS can be used to provide a clean 90° angle between the suture lines.



Prepare the Apical Chamber for Assembly

- Remove the RETAINER RING and coverslip (or OPEN BATH COVER) from the APICAL CHAMBER (see image below).
- Flip the APICAL CHAMBER over and apply a thin film of vacuum grease to flat underside of the chamber.

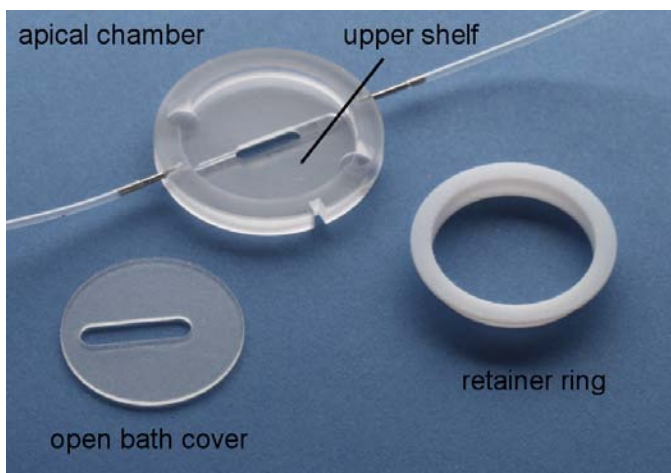
NOTE: Avoid getting vacuum grease into the oval aperture.

Form the Ussing Chamber

- Retrieve the BASOLATERAL CHAMBER from the petri dish, dry the underside, and place it onto a clean surface.
- Orient the APICAL CHAMBER to the BASOLATERAL CHAMBER by aligning the perfusion ports between the two sections.

NOTE: A guide pin in the BASOLATERAL CHAMBER is provided to assure proper orientation between the BASOLATERAL and APICAL CHAMBERS.

- Form the Ussing Chamber by gently pressing the APICAL CHAMBER onto the tissue spanning the aperture in the BASOLATERAL CHAMBER. Use only enough force to provide a secure seal between the two chamber halves.



Seal the Ussing Chamber

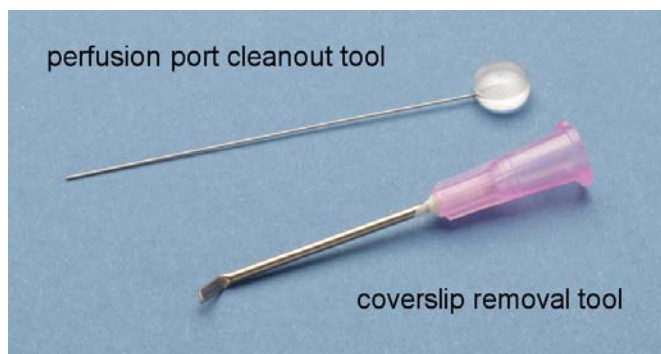
- Using the paintbrush, apply a thin layer of vacuum grease to the upper shelf on the APICAL CHAMBER.

NOTE: Avoid getting grease into the perfusion ports or flow channels which run from the ports to the oval aperture.

14. Press a clean 25 mm round coverslip into the indentation to evenly distribute the grease. Remove the coverslip and dispose. Carefully clean any excess grease from around the aperture and flow channels.

NOTES: A COVERSLIP REMOVAL TOOL has been provided to aid in removal of coverslips. If the perfusion ports become clogged, clear them with the PERFUSION PORT CLEANOUT TOOL.

(See **Maintenance**, page 5).



15. If configuring the **RC-50** for *closed bath* use, then place a clean 25 mm coverslip onto the upper shelf and secure using the RETAINER RING.
16. If configuring the **RC-50** for *open bath* use, then replace the coverslip in step 15 with the OPEN BATH COVER (see page 3).

NOTE: Be sure to align the asymmetric geometry of the OPEN BATH COVER to the associated cutout on the APICAL CHAMBER upper shelf.

Attach Perfusion lines

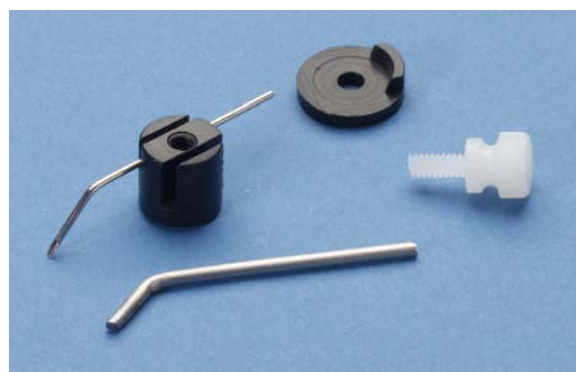
17. Attach input and output perfusion lines to the APICAL and BASOLATERAL CHAMBERS. The *upper perfusion ports* are connected to the APICAL CHAMBER and the *lower perfusion ports* connect to the BASOLATERAL CHAMBER.

NOTES: Pre-fill perfusion lines before making connections. Use care to not disturb the seal between the chambers and sample when making connections. Perfusate is delivered to the **RC-50** chamber through the supplied **PE-90** tubing. **PE-90** tubing fits neatly inside **PE-160** tubing. This allows the **RC-50** to be used with in-line heaters such as Warner's **SH-27B**.

18. If configured for *closed bath* use, connect input and output perfusion lines to both the BASOLATERAL and APICAL CHAMBERS. The direction of fluid flow within the chambers is not important and is left to the user's discretion.
19. If configured for *open bath* use, connect input and output perfusion lines to the BASOLATERAL CHAMBER and an input perfusion line to the APICAL CHAMBER. The direction of fluid flow within the BASOLATERAL CHAMBER remains arbitrary.

NOTE: Connect the apical input perfusion line to the perfusion port furthest away from the bath cutout.

20. If configured for *open bath* use, place a SUCTION TUBE ASSEMBLY (shown to the right) into the output side of the bath. Connect the suction tube to an aspirator.



Mount the Chamber into the Platform

21. Place the assembled Ussing chamber into the platform and tighten the pressure plate onto the place using the pressure plate wrench. A completed open bath assembly is shown on the next page. Note the position of the SUCTION TUBE ASSEMBLY.

MOUNT ONTO THE MICROSCOPE

The **RC-50** chamber can be mounted directly onto a microscope stage if the stage is both flat and has a cutout which fits the platform. In most cases, however, the stage cutout differs from the platform geometry necessitating the use of a stage adapter. Warner Instruments stocks stage adapters for most popular microscopes (see Appendix A) and we will custom manufacture adapters for special applications. Contact our Sales Department for details.



FLUID CONTROL

The selection of solution sources can be of either manual or automatic design and is left to the user. However, Warner Instruments manufactures several perfusion control systems (such as the valve-driven **VC-8** and **VC-8M Control Systems**) which can be used with this application.

Solution delivery can be of a pumped or gravity feed design. In general, a pumped approach will best serve solution delivery and removal for the APICAL and BASOLATERAL CHAMBERS in the closed bath configuration.

The use of a pump is also recommended for solution delivery and removal for the BASOLATERAL CHAMBER in the open bath configuration. Solution delivery to the APICAL CHAMBER can either be pumped or gravity fed since the perfusate is removed by aspiration.

PLATFORM HEATING

Monitoring the heat

Heat is transferred to the platform from a pair of 20 Ω power resistors, mounted on the underside of the platform. Platforms are supplied with a thermistor assembly. The temperature of the platform is monitored by measuring the platform thermistor resistance and adjusting the voltage to the heaters.

Automatic heat control can be achieved by using either a Warner **TTC-324T** or **TTC-344** Temperature Controller (single or dual channel models, respectively). These devices allow the platform or a solution thermistor to be selected as the control sensor. The desired temperature is set and automatically maintained at less than 1°C deviation.

MAINTENANCE

Perfusion port cleaning

The PERFUSION PORT CLEANOUT TOOL (shown on page 3) can be used to clear the perfusion ports if they become clogged with either protein or vacuum grease. The tool may need to be applied from *both ends* of the port since the flow channel has two turns in it. Care must be taken when cleaning these turns to insure that the small plugs placed during fabrication are not removed. If the plugs are accidentally removed, they can be readily re-sealed using RTV (Corning 732).

Note: The **RC-50** perfusion ports should be cleaned immediately after each use.

General cleaning

Cleaning of all **RC-50** components can be performed using a dilute detergent solution. Alternatively, Warner instruments has developed a trisodium phosphate (TSP) wash protocol which is effective in cleaning plastic parts. Contact our Technical Support staff or download the protocol in PDF format from our website.

NOTE: Do not use alcohol, ether or other solvents on plastic parts. Solvents may be used on the anodized surfaces of the platforms. Aluminum chamber parts may also be autoclaved.

APPENDIX

A. Warner Stage Adapters

Microscope Manufacturer	Warner Instrument Stage Adapter Model No.
Nikon Diaphot, TE 200 & TE 300	SA-30 NIK
Nikon TMS with 8 x 12 cm stage cutout	SA-30 TMS8
Nikon TMS with 9 x 13 cm stage cutout	SA-30 TMS9
Olympus IMT (older model)	SA-30 OLY
Olympus IMT-2, IX, & BX50WI	SA-30 OLY/2
Zeiss Axiovert, Leica (Lietz) DMIRB & DMIL	SA-30 LZ
Leica (Lietz) DMIRB/E with 3-plate	SA-30 L3P
Zeiss K stage	SA-30 KZ
Prior and Ludl motorized stages on inverted stages	SA-30 PLI

Note: Warner Instrument **Series P** platforms are designed to fit the Zeiss 76x26 microscope slide frame (#471719) without a stage adapter. Heater platforms will require an insulating material between the platform and frame.

B. Comments

- 1) Silicone vacuum grease (also called stopcock grease) is available from Warner Instruments. (Warner model **111**)
- 2) Best temperature regulation is achieved by preheating the perfusion solution with an in-line heater (Warner model **SH-27B** or **SF-28**) in addition to warming the chamber.